Applicant: James C. Liao Attorney's Docket No.: 06497-013002

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## Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

## **Listing of Claims**:

- 1. (currently amended) A bacterial host cell that produces a heterologous metabolite, the cell comprising a nucleic acid sequence comprising a promoter and nucleic acid sequence encoding a heterologous polypeptide biosynthetic enzyme for production of the heterologous metabolite; the nucleic acid sequence being operably linked to the promoter which is controlled by a response regulator protein; the host cell being genetically modified by deletion or inactivating mutation of a gene encoding a histidine protein kinase having specificity for the response regulator protein such that the promoter is regulated by acetyl phosphate in the absence of nitrogen starvation.
  - 2. (original) The host cell of claim 1 wherein the bacterial cell is an E. coli cell.
- 3. (original) The host cell of claim 1 wherein the promoter is controlled by a response regulator protein selected from the list consisting of ntrC, phoB, phoP, ompR, cheY, creB, and torR.
  - 4. (original) The host cell of claim 3 wherein the promoter is bound by ntrC.
  - 5. (original) The host cell of claim 4 wherein the promoter is glnAp2.
  - 6. (cancelled)
- 7. (original) The host cell of claim 6 wherein the histidine protein kinase is encoded by glnL.

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8. (cancelled)

- 9. (currently amended) A <u>bacterial</u> host cell comprising a first expression cassette comprising a promoter and a nucleic acid sequence encoding a first enzyme <u>required for that</u> <u>catalyzes</u> biosynthesis of a heterologous metabolite; the nucleic acid sequence being operably linked to the promoter which is regulated by acetyl phosphate in the absence of nitrogen starvation; and nucleic acid sequences expressing other enzymes <u>required for that catalyze</u> biosynthesis of the metabolite.
  - 10. (original) The host cell of claim 9 wherein the metabolite is an isoprenoid.
  - 11. (original) The host cell of claim 10 wherein the isoprenoid is a carotenoid.
- 12. (original) The host cell of claim 10 wherein the isoprenoid is lycopene, β-carotene, astaxanthin, or one of their precursors.
- 13. (original) The host cell of claim 10 wherein the first enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, or 1-deoxyxylulose 5-phosphate synthase.
- 14. (original) The host cell of claim 9 wherein the first enzyme is phosphoenolpyruvate synthase.
  - 15. (cancelled)
- 16. (currently amended) The host cell of claim  $\frac{15}{9}$  wherein the bacterial cell is an  $E.\ coli$  cell.

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17. (currently amended) The host cell of claim 15 9 wherein the cell is lacking a functional histidine protein kinase gene.

- 18. (currently amended) The host cell of claim 15 9 wherein the promoter is controlled by ntrC, phoB, ompR, cheY, creB, phoP, or torR.
  - 19. (original) The host cell of claim 18 wherein the promoter is bound by ntrC.
  - 20. (original) The host cell of claim 19 wherein the promoter is glnAp2.
- 21. (original) The host cell of claim 10 wherein the host cell further contains a second expression cassette comprising a nucleic acid sequence encoding a phosphoenolpyruvate synthase operably linked to a promoter which is regulated by acetyl phosphate concentration.
- 22. (withdrawn) A method of producing <u>a</u> heterologous isoprenoid [[s]] in a host cell, <u>the method</u> comprising:

providing the host cell of claim 9, wherein the first enzyme is a biosynthetic enzyme that catalyzes synthesis of the heterologous isoprenoid;

overexpressing a heterologous phosphoenolpyruvate synthase; and expressing biosynthetic enzymes required for synthesis of the heterologous isoprenoid.

23. (withdrawn) A method of producing a lycopene in a <u>bacterial</u> host cell, the <u>method</u> comprising:

expressing a heterologous 1-deoxy-D-xylulose 5-phosphate synthase, a heterologous geranylgeranyl diphosphate synthase, a heterologous phytoene synthase, and a heterologous phytoene desaturase, at least one of which is expressed from a coding nucleic acid whose transcription is controlled by ntrC and acetyl phosphate concentration.

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24. (currently amended) A kit comprising (i) a nucleic acid sequence containing a promoter controlled by a response regulator protein such that the promoter is regulated by acetyl phosphate in a defined <u>bacterial</u> host cell; and (ii) the defined host cell which is genetically modified by deletion or <u>inactivating</u> mutation of a histidine protein kinase gene.

- 25. (original) A nucleic acid sequence comprising a promoter and a sequence encoding a biosynthetic enzyme required for that production of a first metabolite, the sequence being operably linked to the promoter which is regulated by a second metabolite whose concentration is indicative of availability of a precursor for the biosynthesis of the first metabolite.
- 26. (original) The nucleic acid sequence of claim 25 wherein the second metabolite is a waste product produced from a precursor for the biosynthesis of the first metabolite.
- 27. (original) The nucleic acid sequence of claim 25 wherein the first metabolite is an isoprenoid.
- 28. (original) The nucleic acid sequence of claim 27 wherein the isoprenoid is a carotenoid.
- 29. (original) The nucleic acid sequence of claim 28 wherein the isoprenoid is lycopene,  $\beta$ -carotene, astaxanthin, or one of their precursors.
- 30. (original) The nucleic acid sequence of claim 25 wherein the second metabolite is acetyl phosphate, cAMP, fructose 1-phosphate, or fructose 6-phosphate.
- 31. (original) The nucleic acid sequence of claim 30 wherein the second metabolite is acetyl phosphate.

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32. (original) The nucleic acid sequence of claim 31 wherein the promoter is controlled by ntrC, phoB, ompR, cheY, creB, phoP, or torR.

- 33. (currently amended) The nucleic acid sequence of claim 32 31 wherein the promoter is bound by ntrC.
  - 34. (original) The nucleic acid sequence of claim 33 wherein the promoter is glnAp2.
- 35. (original) The nucleic acid sequence of claim 27 wherein the biosynthetic enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, or phosphoenolpyruvate synthase.
- 36. (new) The host cell of claim 1 wherein the cell is a *Bacillus subtilis*, *Salmonella typhimurium*, *Agrobacterium tumefaciens*, *Thermus thermophilus*, or *Rhizobium leguminosarum* cell.
- 37. (new) The host cell of claim 1 wherein the heterologous metabolite is a polyketide.
- 38. (new) The host cell of claim 1 wherein the heterologous metabolite is a polyhdroxyalkanoate.
  - 39. (new) A bacterial host cell comprising:
    - (i) a genetic alteration inactivating the glnL gene; and
- (ii) a nucleic acid sequence comprising a coding sequence encoding a phosphoenol pyruvate synthetase (pps) and an operably linked promoter that is regulated by ntrC and acetyl phosphate.

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40. (new) A bacterial host cell comprising:

- (i) a genetic alteration inactivating the glnL gene; and
- (ii) a nucleic acid sequence comprising a coding sequence encoding a biosynthetic enzyme that catalyzes production of an isoprenoid, polyketide, or polyhdroxyalkanoate, and an operably linked promoter that is regulated by ntrC and acetyl phosphate.
- 41. (new) The host cell of claim 40 wherein the biosynthetic enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, or phosphoenolpyruvate synthase.
  - 42. (new) The kit of claim 24 wherein the defined host cell is an E. coli host cell.
- 43. (new) The kit of claim 42 wherein the histidine kinase gene is glnL and the response regulator is ntrC.
- 44. (new) The kit of claim 42 wherein the nucleic acid further comprises a restriction enzyme polylinker located 3' of the promoter such that transcription of a sequence inserted into the polylinker is controlled by the promoter.
  - 45. (new) The kit of claim 42 wherein the promoter is the glnAp2 promoter.
- 46. (new) A method of producing a heterologous metabolite in a bacterial host cell, the method comprising:

providing the host cell of claim 1; and culturing the host cell under conditions such that acetyl phosphate triggers the promoter.

47. (new) The method of claim 46 in which the culturing comprises nitrogen rich conditions.

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48. (new) The method of claim 46 in which the culturing comprises growth to late logarithmic growth or stationary phase.